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[*Authors alone are responsible for all opinions expressed in their Communications.*]

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No. 39.

STUDIES ON THE OXIDISING ACTION OF SELENIOUS ACID.

I. ORGANIC SULPHUR COMPOUNDS.

By A. E. A. WERNER.

[Read March 25. Published separately April 30, 1941.]

It has long been known that selenious acid and selenites are readily reduced to selenium by a large variety of chemical compounds, and recently Riley and his co-workers (1) in a series of papers have demonstrated the specific oxidising action of selenious acid towards a number of organic compounds, including aldehydes, ketones, alcohols, unsaturated hydrocarbons, and esters. Further work by Mel'nikov (2), who has shown that mercaptans can be reduced to disulphides, and a recent test for the detection of selenites, devised by Deniges (3), which depends on the production of a red precipitate of selenium by the addition of thiourea to the acidified solution of the selenite, has led to the present investigation of the oxidising action of selenious acid on a variety of organic compounds containing sulphur, including thiourea and its derivatives, thioamides, thioacids, mercaptans, and thiophenols. All these compounds either contain the easily oxidisable –SH group present in the molecule, or can give rise to it by a simple intramolecular change, so that the oxidising action of the selenious acid can be represented by the following general equation :—

$$4\ R\text{–}SH + H_2SeO_3 = 2\ R\text{–}S\text{–}S\text{–}R + Se + 3\ H_2O.$$

This reaction can be used as a specific test for the detection of the presence of the –SH group in organic sulphur compounds, because a red precipitate of selenium is always produced if the experimental conditions are suitably chosen, as will be discussed later. Furthermore, the different experimental conditions under which the reaction can be carried out provide valuable information concerning the molecular structure of the compounds under investigation.

The actual results obtained are best considered under the following headings :—

A. *Thiourea and Derivatives.* When selenious acid is added to an aqueous solution of thiourea, there is an immediate precipitate of selenium; in fact, the test devised by Deniges (*loc. cit.*) for the detection of selenites can serve equally well as a sensitive test for thiourea, since a distinct reddish turbidity due to colloidal selenium is produced with one part of thiourea in 50,000 parts of water. The author (4) has also evolved a method for the quantitative estimation of thiourea based on this reaction.

It is now found that all mono-, di-, and tri-substituted derivatives of thiourea react with selenious acid, producing either a *red* precipitate of selenium or an *orange* precipitate consisting of selenium contaminated with sulphur. It was

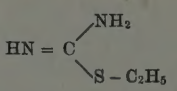
also found that the velocity of the reaction varies with the different derivatives, being instantaneous in the case of the parent thiourea, and decreasing progressively as one precedes from mono- to tri-substituted derivatives.

The essential results obtained may be summarised in the following table :—

TABLE. 1.—Reaction of substituted Thioureas with Selenious acid.

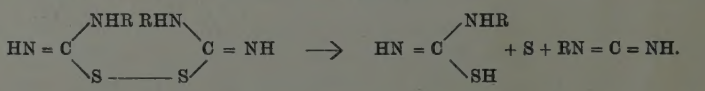
Substance tested.	Nature of Reaction.
Methyl thiourea ... ... / Allyl thiourea	Immediate red ppt. of selenium.
Phenyl thiourea	Immediate orange ppt. of selenium and sulphur.
Sym-Diethyl thiourea ...	Slow reaction. Orange ppt.
Trimethyl thiourea ... ... / Diethylbenzyl thiourea ... / Dimethylphenyl thiourea ...	Very slow reaction. Orange ppt. of selenium and sulphur.

In the case of tetrasubstituted derivatives of thiourea or substituted isothioureas, e.g., ethyl isothiourea of the formula



no reaction with selenious acid can be observed.

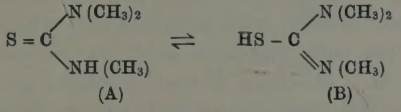
The sulphur with which the selenium precipitate is in many cases contaminated is derived from the decomposition of the resulting formamidine-disulphide according to the following equation :—



The extent to which this decomposition takes place depends, firstly, upon the nature of the substituent groups present in the thiourea molecule, and, secondly, upon the degree of acidity of the solution; the more strongly acid the solution the less is the amount of this decomposition. In the presence of excess strong mineral acids the formamidine-disulphides are comparatively stable, but decompose when these solutions are diluted with water. In agreement with this fact it was found, in the case of those substituted thioureas which gave an *orange* precipitate in weakly acid solution, that if the reaction were carried out in a solution of concentrated hydrochloric acid a deep *red* precipitate of selenium was rapidly formed quite free from any sulphur. The stability of substituted formamidine-disulphides in the presence of strong mineral acid is also indicated by the work of Fichter and Braun (5), who subjected various substituted thioureas to electrolytic oxidation, and succeeded in isolating the resulting formamidine-disulphides as salts with perchloric acid.

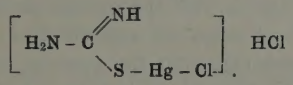
In the present case formamidine-disulphides are in fact the main product of the oxidising action of selenious acid on thioureas, as can readily be shown by the isolation from the filtrate of the insoluble salts formed on the addition of a saturated solution of picric acid.

The results presented in Table 1 are best interpreted on the assumption that reduction of the selenious acid occurs provided there is present in the substituted thiourea molecule a free hydrogen atom which can migrate from nitrogen to sulphur, thus enabling the iso-thiourea structure containing the vulnerable –SH group to be attained. Thus, in the case of trimethylthiourea, for example, the following equilibrium is possible between the thiocarbamidic structure (A) and the iso-thiourea structure (B) :—

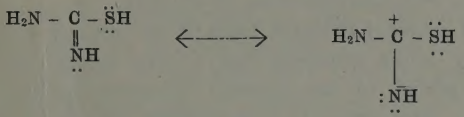


The molecular form (B) reacts with selenious acid as rapidly as it is formed, so that the velocity of the reaction is governed by the extent to which the isomeric change A → B takes place. This change in turn is influenced greatly by the nature of the solvent, being favoured by a moderate increase in acidity.[1]

The fact that thiourea and its derivatives can exist in the iso-thiourea form is well known from the work of Werner (6) on the action of nitrous acid on thiourea, and also from the results of Ray (7) on the reaction between thiourea and mercuric chloride, in which a compound of the formula



is formed. Recently Clow (8) has produced evidence on a purely physical basis, namely, from measurements of the magnetic susceptibility, that thiourea (in the solid state) exists wholly as an amino-imino hybrid involving resonance between the two electronic structures,



whereas the derivatives of thiourea exist as a mixture of this amino-imino hybrid and the true thiocarbamidic form in varying proportions, depending upon the nature of the substituent group.

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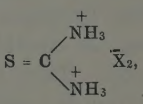
[1] The isomeric change also readily occurs in alkaline solution, but since only the free selenious acid acts as an oxidising agent there is no reaction when a selenite is added to an alkaline solution of thiourea.

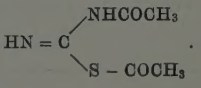
Finally, this hypothesis that the isomeric change postulated above does actually occur in the reaction between selenious acid and the different substituted thioureas is confirmed by carrying out the reaction under experimental conditions of extreme acidity. For, in the presence of an excess of a very strong mineral acid, the thiourea molecule will be present as the salt of the formula



in which the vulnerable –SH group is not only absent but cannot arise by a simple isomeric change. In agreement with this view it was found that thiourea and its derivatives do not react with selenious acid when they are dissolved in such solvents as 50 per cent. sulphuric acid, 60 per cent. perchloric acid, or syrupy phosphoric acid. However, if these solutions are diluted with distilled water the reduction reaction starts, and the characteristic precipitate of selenium is formed. Furthermore, it is worthy of note that in the case of the parent thiourea itself the reduction reaction starts immediately after the addition of water, but in the case of the various derivatives of thiourea the precipitation of selenium takes place slowly at a rate determined by the velocity with which the isomeric change between the carbamidic and the iso-thiourea structures occurs.

The behaviour of the acyl derivatives of thiourea towards selenious acid was similar to that of the alkyl derivatives. Thus, monoacetyl and monobenzoyl thiourea both react very slowly in aqueous solution, or in the presence of a weak acid such as acetic acid, with the formation of an orange-yellow precipitate consisting of a mixture of selenium and sulphur; however, in the presence of a large excess of hydrochloric acid the reaction is not only exceedingly rapid, but also the precipitate formed is red in colour, and contains no sulphur. These results are best interpreted on the assumption that the monoacyl derivatives of thiourea are only converted very slowly in the absence of a strong mineral acid into the iso-thiourea form, and, secondly, that the resultant acyl formamidine-disulphide is very unstable in aqueous or weakly acid solution, and readily decomposes with the liberation of sulphur.

In the case of diacetyl thiourea there is no reaction with selenious acid in aqueous or weakly acidic solutions, but a very rapid reaction occurs if the selenious acid is added to the diacetyl thiourea dissolved in concentrated hydrochloric acid. This result is in agreement with the formula proposed by Werner (9) for diacetyl thiourea, namely,

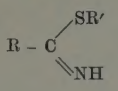


In the presence of a strong acid the acetyl group attached to the sulphur atom is readily removed by hydrolysis with the formation of monoacetyl thiourea,

which reacts as previously described. Finally, it may be noted that in the strongly acid solvents, such as 60 per cent. perchloric acid, these acyl derivatives behave similarly to the alkyl derivatives, and do not react with selenious acid until the solution is diluted with water.

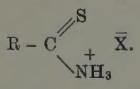
B. *Thioamides.* Thioacetamide and thiobenzamide were chosen as typical representatives of this class of organic compounds; they were found to react immediately with selenious acid in aqueous solution or in glacial acetic acid, with the formation of an orange precipitate consisting of selenium mixed with a small amount of sulphur. This result is in agreement with the fact that thioamides can exist as an equilibrium mixture of the true thioamide form and the corresponding iso-thioamide form, namely,

Evidence for this equilibrium has been produced from various sources, in particular the formation of S-ethers,



with alkyl halides.

In the presence of strong acid in excess the formation of the acidic iso-thioamide form is completely suppressed in favour of the basic thioamide form, which will be converted into the salt



This agrees with the experimental fact that in such solvents as 60 per cent. perchloric acid no reaction occurs on the addition of selenious acid, until the solution is diluted with water, when there is an immediate red precipitate of selenium.

C. *Thioalcohols and Thioacids.* The behaviour of these two classes of compounds is in direct contrast to that of the other sulphur compounds so far studied. In alcohol, dilute acid solution, or even when dissolved in glacial acetic acid, such compounds as ethyl mercaptan, thiophenol, thioglycollic acid, thiosalicylic acid, or cysteine, do not react with selenious acid; in the case of the thioacids a white crystalline mass precipitates out, which consists of a complex of the thioacid and selenious acid in molecular proportions. If this complex is dissolved in *concentrated* hydrochloric acid it decomposes with the precipitation of selenium, and formation of the corresponding disulphide acid. Furthermore, if these compounds are dissolved in very strongly acid solution, such as 60 per cent. perchloric acid or syrupy phosphoric acid, there is an immediate reaction on the addition of selenious acid, and a copious red precipitate of selenium is formed. This marked difference in behaviour may be due to the fact that in these thioalcohols and thioacids the sulphydryl group

is not so readily oxidised, as is the case with the thiourea and thioamide derivatives, unless the experimental conditions are especially favourable; apparently the most favourable conditions are those of extreme acidity. In this connection it is of interest to consider the reactivity of thioacids towards other oxidising agents. Thus, Schöberl and Krumey (10) have noted that the ease with which phosphotungstic acid is reduced by various thioacids depends upon the pH of the solution; also Schöberl and Rambacher (11) have found that the rate of splitting of the –S–S– linkage by sodium sulphite or potassium cyanide varies in different compounds, and depends on the pH of the solution. Furthermore, Schöberl (12) has found that *aa*-dimethyl- and diphenylthioglycollic acids were not oxidised by iodine in acid solution, whereas the primary thioglycollic acid and the secondary monomethyl-thioglycollic acid were both readily oxidised.

From the above results it is clear that the precipitation of selenium on the addition of selenious acid to sulphur-containing organic compounds under the appropriate experimental conditions might be used as a sensitive and specific test for the presence of the –SH group. In order to test this conclusion a large variety of other sulphur-containing organic compounds were treated with selenious acid, but in no case was there any precipitate of selenium formed. The compounds tested included:—(*a*) thioethers, e.g., diethyl- and diallyl-sulphide; (*b*) disulphides, e.g., dithiosalicylic acid; (*c*) cyclic sulphur compounds, e.g., thiophene, pseudothiohydantoin; (*d*) compounds containing the $> C = S$ group, e.g., isothiocyanates, diphenylthiohydantoin, tetramethyl-thiuramdisulphide.

It may be, therefore, taken as established that the precipitation of selenium on the addition of selenious acid constitutes a specific test for the presence of the sulphydryl group in organic sulphur compounds.

This communication is concerned mainly with the qualitative nature of the reaction between selenious acid and sulphur organic compounds, but it is hoped in a later communication to investigate this reaction in greater detail and on a quantitative basis.

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No. 40.

THE EFFECT OF WAVE-LENGTH ON THE RELATION BETWEEN THE INTENSITY OF ILLUMINATION AND THE CURRENT IN SELENIUM RECTIFIER PHOTO-CELLS.

By H. H. POOLE and W. R. G. ATKINS, F.R.S.

[Read March 25. Published separately July 14, 1941.]

In the course of standardising selenium rectifier photo-cells for use under water we found that the departure from linearity in the response of the cells in intense light was much greater in the deep red than in other parts of the spectrum (1). This curvature of the light/current characteristic increases with the resistance of the measuring circuit, and is generally attributed to the effect of the potential difference produced by the primary photo-electric transfer of electrons from the selenium to the overlying metal film which forms one electrode of the cell. This potential difference, which for a given illumination increases with the external resistance, drives some of the electrons back again, this leakage current increasing more rapidly than the voltage, with consequent reduction of the external current. Atkinson, Campbell, Palmer, and Winch (2) have pointed out that this cannot be a complete explanation, since their work shows, in confirmation of that of Büchmuller and König (3), that for moderate illuminations and low external resistances the effect may be reversed, the current increasing more rapidly than the voltage.

It seemed to us to be probable, however, that the greater fall in sensitivity with increasing current which we had observed with low-resistance circuits in red light was due to increased back-leakage, and we tentatively suggested that it might be due to the photo-conductivity of selenium, which has its maximum in the red part of the spectrum.

To examine the matter further a new photometer bench has been made, about 3·5 m. long and carrying at one end a 250-watt 220-volt projector lamp mounted in a dark lantern from which the light only emerged through a window of suitable size along the axis of the bench. The cells to be tested or compared were mounted on a carrier with a transverse slide, so that either could be brought into the exposing position behind the desired colour filter, the cell not in use being protected by an opaque screen. The cells were adjusted on the carrier so that the sensitive surface of whichever cell was exposed was in the same position relative to the carrier, and the distance of this surface from the plane of the lamp filament was set at some definite distance by sliding the carrier along the bench until it came against a suitable distance-piece interposed between it and a fixed block, which carried a large water cell interposing a thickness of 4·4 cm. of water in the path of the light beam. It was found that

with the carrier in contact with the block the distance of the cell surface from the plane of the filament was 13·7 cm. Refraction in the water cell reduces the effective optical distance to 12·45 cm. The cells tested were nearly 4 cm. in diameter, and the lamp filament approximately occupied a vertical rectangle about 1·2 cm. × 1·1 cm., so as an allowance for the finite sizes of source and cell 4·0 sq. cm. was added to the square of the optical distance when finding the lengths of the distance-pieces needed to give the desired relative illuminations. This was probably a slight over-correction (5), but as it only amounted to about 2·5 per cent. at the shortest distance any such error should not be serious. The distance-pieces were of such lengths that, when allowance was made for the water cell and for the sizes of source and photo-cell, each successive distance-piece or combination of distance-pieces reduced the light to 1/2. Thus if I were the illumination without any distance-piece—say about 15,000 lux (without filter)—the other values used were $I/2$, $I/4$, etc., down to $I/512$, with a final reduction factor of $I/\sqrt{2}$ to $I/725$, or about 20 lux. The filters used of course reduced the effects of these illuminations by different amounts. The long range and the large illuminations required rendered it impracticable to use the much more accurate additive method used for short ranges, such as 10 to 1, by Atkinson, Campbell, Palmer, and Winch.

The bench, which was carefully blackened, was set up in a dark room, and fixed and movable diaphragms prevented any appreciable amount of reflected or scattered light from reaching the cell. A shutter mounted close to the water cell protected the cell from light except during the few seconds while a reading was being taken, so that the effects of fatigue, or of the warming of the cell by the radiation, were minimized.

The lamp current was provided by a shunt-wound D.C. generator driven at very nearly constant speed by a three-phase motor, the voltage across the lamp being maintained as nearly as possible at 210 volts by hand regulation of a series rheostat. The photo-electric current was measured by the modified Campbell-Freeth balanced circuit which we have used at sea (4), except that a sensitive mirror galvanometer with suitable attenuator was used for balancing instead of the interrupter, amplifier, and telephone, and that an additional resistance of 1000 ohms could be inserted when required in series with the cell, thus changing the effective external resistance of the cell circuit from zero to 1000 ohms. The resistance controlling the balancing current ranged from 2,000 to 200,000 ohms, and the E.M.F. supplied by the potentiometer from a few millivolts to nearly 2 volts.

The first of a set of tests was made at the maximum distance, successive readings being taken with the shutter open and closed, so as to reduce greatly the small effect produced by any stray light scattered by the walls of the room. The tests were repeated with 1000 ohms in series with the cell, and then the second cell was brought into position and tested. The filter could then be changed for another filter or combination, thus giving a rapid comparison of the sensitivities for different spectral regions. The carrier was then moved up to the next position, and the operation repeated, and so on.

The results for two cells of different makes are plotted in figure 1. The relative intensities of illumination for a complete set of tests with a given filter are in the ratios $1/\sqrt{2}$, 1, 2, 4, ... 512, hence we can find the relative values of the sensitivity (i.e. current/illumination) and express them as fractions of the sensitivity of the given cell and filter at the minimum illumination. As the only method of comparing the illuminations under different filters is by the resultant currents, which vary over a very wide range, the logarithm of this current is taken as abscissa. It may be noted that the Electrocell is considerably more sensitive than the Weston, as shown by the larger values of

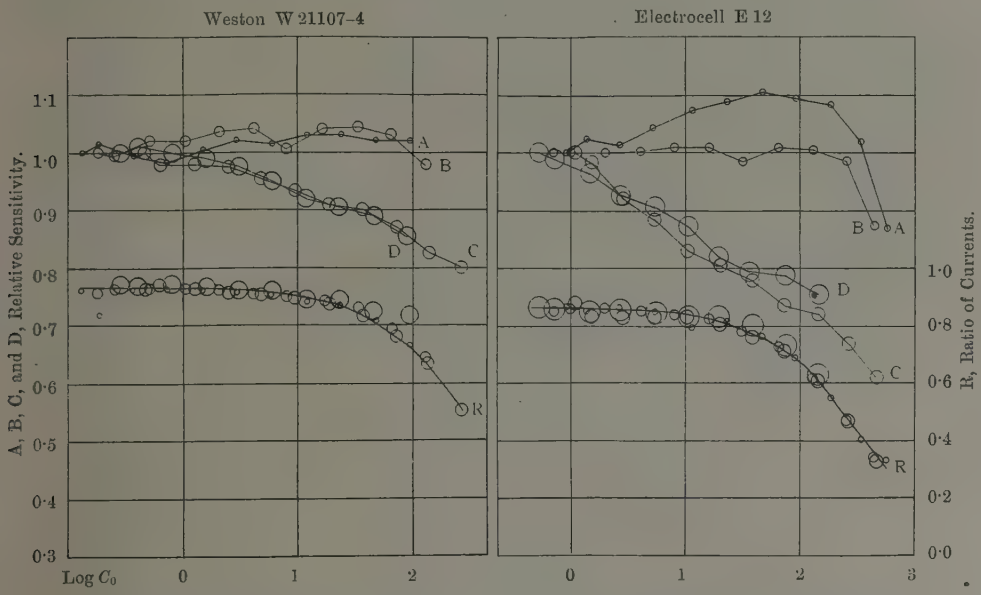

FIG. 1.—Variation with current of the sensitivity for zero external resistance and of the effective internal resistance of selenium cells when illuminated by light of various wave-lengths. *A*. Sensitivity with Schott BG 12 filter, about 3600 to 5000 Å. *B*. Sensitivity with Schott RG 1 and Corning Green filters, about 6000 to 6600 Å. *C*. Sensitivity with Schott RG 5 filter, over 6600 Å. *D*. Sensitivity with Schott RG 5 and Zeiss 966/8 filters, over 7000 Å. *R*. Ratio of current through external resistance of 1000 ohms to current for same illumination through zero resistance. The same symbols are used for plotting the corresponding points as for the sensitivity curves. There is in this case, however, no significant difference between the results for different spectral bands. C_0. Current in microamperes for zero external resistance.

the current corresponding to the same bench settings. It is also relatively more blue-sensitive, as may be seen by comparing the maximum currents for curves *A*, *B*, and *C* for each cell. One reading in curve *B* is appreciably low for each cell. As these two readings were taken successively at the same bench setting it would seem that there must have been some small error in this, or that the voltage of the lamp may have fallen temporarily.

Both cells showed the initial increase of sensitivity with current through the zero-resistance circuit, as found by other workers, the Electrocell for blue light only, the Weston for both blue and orange-red. With deep red, however, the effect is quite different, and the comparatively sudden change near 6600 Å is very striking. There is no evidence that further increase of wave-length produces any further change, but curves *D* are not quite comparable with the others, as the small size of the only filter available having a cut-off about 7000 Å necessitated a modification in the arrangement, the filter being placed in a fixed position close to the water cell with the RG 5 filter in the standard position on the carrier to reduce the effect of any scattered light. This arrangement prevented the carrying out of measurements at the minimum distance attainable with the other filters.

The curves *R* show the ratios of the currents through 1000 ohm and zero-resistance circuits, and it is evident that apart from a few irregular points these fall fairly well on a smooth curve for each cell, regardless of what filter is used. Thus the change in curvature which sets in near 6600 Å is not due, as we had previously supposed, to an increase in the leakage current through the cell, as this would have been apparent as a fall in value of this ratio.

Figure 2 is plotted from results we obtained some years ago for a Weston cell, and it may be significant that the change of curvature which we have found

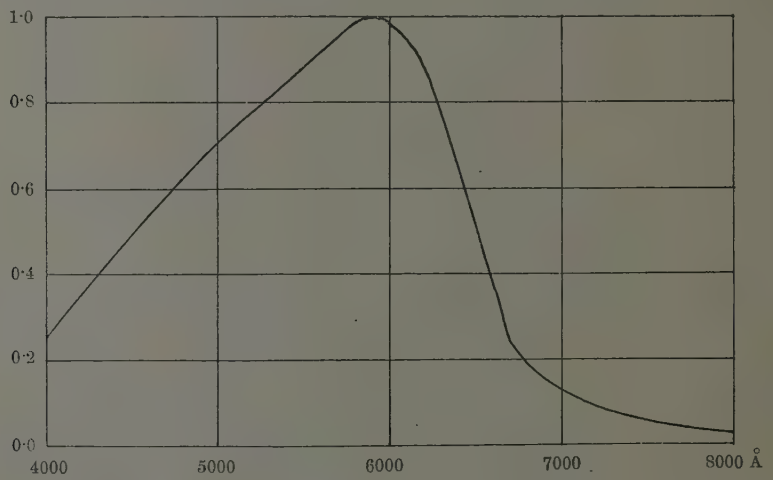

Fig. 2.—Relative spectral sensitivity of Weston selenium cell.

occurs about the region where the fall in sensitivity with increasing wave-length is at its steepest. It seems possible that a rectifier cell may possess more than one threshold, so that, starting with waves too long to energise any electrons in the selenium and enable them to cross the boundary into the metal, we may,

as the wave-length is reduced, affect successively electrons in different bands of energy levels. If we suppose that one such threshold occurs near 6600 Å there may be a considerable difference in the numbers of electrons responsive to light according as the wave-length is above or below that value. If we further suppose that the comparatively large currents generated in rectifier cells in bright light are sufficient to affect the equilibrium, and reduce appreciably the numbers of electrons in the upper energy levels, we would get a tendency to saturation in the current, which would be considerably more pronounced for wave-lengths above 6600 Å, as we observe. We would not expect to find any thresholds further down in the visible spectrum, since the sensitivity after reaching a maximum near 5900 Å falls again, this fall being possibly due to a combination of such causes as the reduction in the number of quanta per erg, a reduction with wave-length of the probability of an electron acquiring energy from a quantum, and/or a reduction in the percentage of the incident light which is transmitted by the overlying metal film. It is worth noting that the Electrocell, which is relatively more blue-sensitive than the Weston, and may have a maximum sensitivity further down the spectrum, shows curve *B* as intermediate between *A* and *C*, whereas for the Weston there is no significant difference between *A* and *B*. This may mean that for the Electrocell the threshold is rather below that for the Weston.

This attempt at an explanation does not account for the rise of sensitivity with illumination where the latter is moderate and of sufficiently short wave-length, and the external resistance is low. This effect, however, does not seem to be apparent in all cells, and may be due to some secondary cause. The increased fall in sensitivity with illumination in deep red light was found in every cell which we tested, namely, eight Electrocells and two Westons, so it seems likely to be a general property of selenium rectifier cells.

SUMMARY.

(1) Photometer bench tests of selenium rectifier cells in illuminations of different colours and covering a range of 725 to 1 for each colour show that the fall in sensitivity (i.e. current/illumination) with increasing illumination was greater for wave-lengths exceeding 6600 Å than for other spectral regions. The currents were measured by the Campbell-Freeth balanced circuit giving the effect of a galvanometer of zero resistance.

(2) When a resistance of 1000 ohms was included in the measuring circuit the resultant relative fall in the current for a given illumination was a function of the zero-resistance current only, and, for a given value of this, was independent of the colour of the light.

(3) It would seem, therefore, that the increased fall in sensitivity with illumination found in deep red light is not due to increased leakage of current in the cell, as the effect of this would be greater with large external resistance.

(4) A tentative suggestion is put forward that the effect may be due to the occurrence of a threshold for the selenium cell in the neighbourhood of 6600 Å, and that the number of electrons possessing sufficient energies to respond to longer wave-lengths may be so restricted as to increase appreciably the tendency towards saturation noticeable in the current.

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No. 41.

ATMOSPHERIC POLLUTION IN DUBLIN DURING THE YEAR 1940.

By A. G. G. LEONARD, BRIDGET P. McVERRY, and D. CROWLEY.

[Read April 22. Published separately July 28, 1941.]

Work has been continued during 1940 at Leinster Lawn and the Albert Farm, Glasnevin.

Results obtained with Standard Gauges.

Detailed figures for deposits at Leinster Lawn and Albert Farm are recorded in Tables 1 and 2 respectively.

Soluble solids were deposited at Leinster Lawn at about 74 tons per square mile, and were 1·8 times the quantity at Glasnevin.

Insoluble solids at Leinster Lawn amounted to 113 tons per square mile, and were 4·2 times the quantity at Glasnevin.

The total solids at both stations show a distinct increase over the figures obtained in previous years.

Suspended Impurity and Sulphur Dioxide.

Results of suspended impurity measurements at Merrion Street by the Owens Automatic Filter, and of sulphur dioxide at Leinster Lawn and the Albert Farm by the lead peroxide method, are given in the following table, the sulphur dioxide being recorded as sulphur trioxide:—

	Average Quantity of Suspended Impurity in Milligrams per cubic metre.	Milligrams of SO_3 per 100 sq. cm. of PbO_2 per diem.	
		Merrion St.	Albert Farm.
Jan.	0·82	3·39	0·82
Feb.	0·37	1·23	0·76
Mar.	0·36	1·10	0·47
Apr.	0·30	—	0·36
May	0·25	0·96	0·26
June	0·16	0·71	0·20
July	0·18	0·79	0·13
Aug.	0·18	0·79	0·13
Sep.	0·21	1·06	0·17
Oct.	0·29	1·74	0·60
Nov.	0·42	2·12	0·44
Dec.	0·47	2·40	0·42

TABLE I.—*Impurity Deposited from the Atmosphere at Leinster Lawn during the Year* 1940.

Grams per Square Decametre (Metric Tons per Hundred Square Kilometres).

—	Rain-fall, mm.	pH of rain-water.	Insoluble Matter.			Soluble Matter.		Total Solids.	Included in Soluble Matter.			
			Tar.	Carbona-ceous other than Tar.	Ash.	Loss on ignition.	Ash.		Sulphates (SO_3).	Chlorine (Cl).	Ammonia (NH_3).	Lime (CaO).
January ...	59	4·4	19	151	252	115	159	696	74	47	3	48
February ...	90	5·0	23	110	281	110	245	769	82	103	3	55
March ...	31	5·7	12	107	235	34	178	566	50	31	2	44
April ...	74	5·9	12	151	227	102	104	596	81	48	3	51
May ...	20	6·2	7	127	216	24	119	493	36	9	—	26
June ...	24	6·6	8	135	330	102	94	669	40	15	—	42
July ...	102	6·2	9	105	192	82	136	524	59	20	—	45
August ...	12	6·6	8	177	219	62	145	611	42	15	—	37
September ...	47	6·5	7	118	245	63	186	619	47	35	—	57
October ..	134	5·2	11	124	232	97	165	629	80	40	3	53
November ...	86	6·1	7	80	233	79	178	577	62	34	2	41
December ...	44	5·4	20	90	225	140	227	702	97	85	4	54
Mean Monthly ...	60	—	12B	123B	241B	84B	161B	621B	65A	40B	2A	46
Summer Total ...	279	—	51	813	1429	435	784	3512	305	142	3	258
Winter Total ...	441	—	92	662	1458	575	1152	3939	445	340	17	295
Annual Total ...	723	—	143	1475	2887	1010	1936	7451	750	482	20	553

TABLE II.—*Impurity Deposited from the Atmosphere at Glasnevin during the Year* 1940.

—	Rain-fall, mm.	pH of water.	Grams per Square Decametre (Metric Tons per Hundred Square Kilometres).								
			Insoluble Matter.			Soluble Matter.		Total Solids.	Included in Soluble Matter.		
			Tar.	Carbona-ceous other than Tar.	Ash.	Loss on ignition.	Ash.		SO_3	Cl.	NH_3
January ...	73	4·45	3	24	45	64	103	239	50	32	7
February ...	58	4·54	2	27	40	74	153	296	39	54	2
March ...	38	5·73	2	31	31	38	67	169	24	14	5
April ...	66	5·20	2	28	39	79	61	210	38	18	7
May ...	29	5·60	1	41	41	40	40	163	26	5	—
June ...	17	6·35	1	81	62	76	39	259	12	6	—
July ...	110	6·18	1	54	45	55	72	226	42	9	1
August ...	11	6·44	1	28	61	38	53	182	23	5	—
September ...	49	5·50	1	30	43	41	70	185	31	14	1
October ...	122	4·98	3	39	40	73	116	271	61	28	7
November ...	82	5·39	2	39	130	46	83	301	45	21	5
December ...	40	5·78	3	22	28	43	124	220	34	52	5
Mean Monthly ...	58	—	2	37	50	56	82	227	35	21	3
Summer Total ...	282	—	7	262	291	329	335	1225	172	57	9
Winter Total ...	413	—	15	182	314	338	646	1496	253	201	31
Annual Total ...	695	—	22	444	605	667	981	2721	425	258	40

The results obtained in January were the highest so far recorded and were due to the lengthy spell of very cold weather in that month, which gave rise to greatly increased coal consumption.

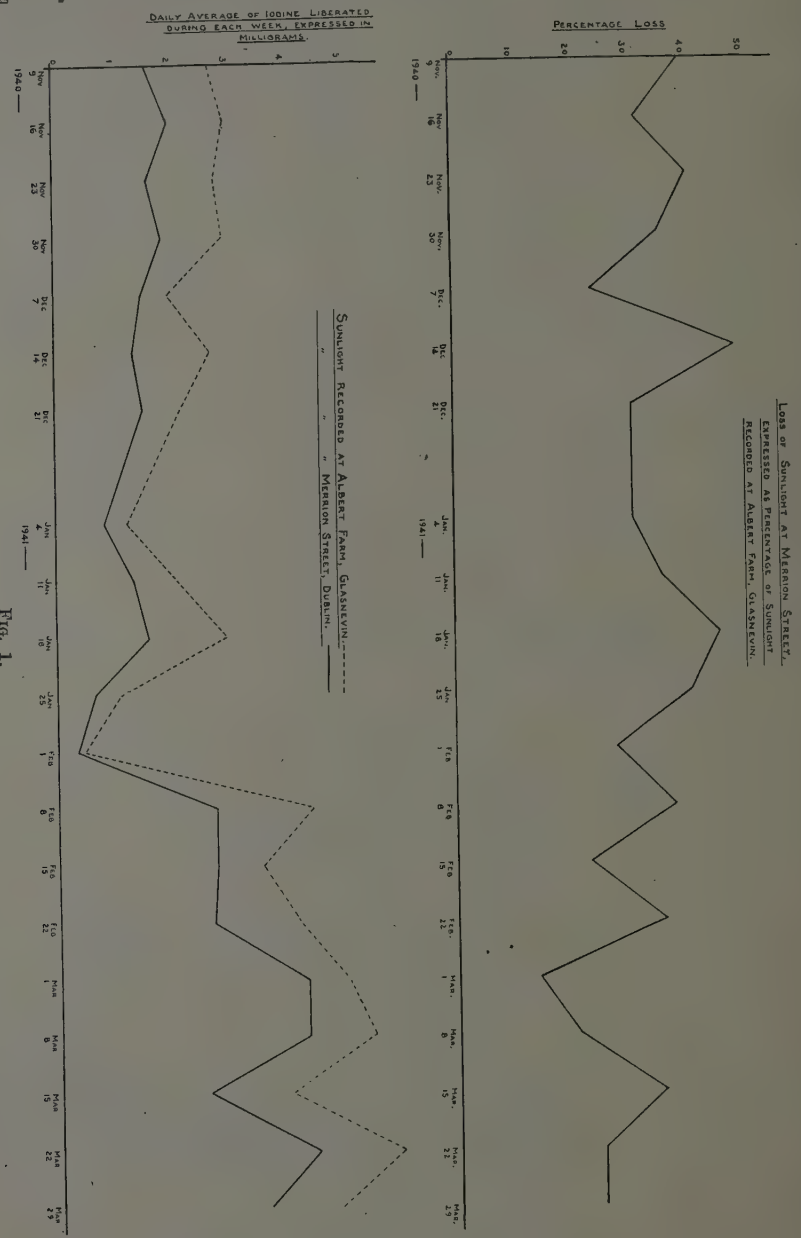
Daily Average of Iodine Liberated during each week, expressed in Milligrams.
Sunlight Recorded at Albert Farm, Glasnevin.
" " Merrion Street, Dublin.
Percentage Loss
Loss of Sunlight at Merrion Street, expressed as Percentage of Sunlight recorded at Albert Farm, Glasnevin.

Fig. 1.

Owing to an accident in the laboratory no result was obtained for sulphur dioxide at Merrion Street in April.

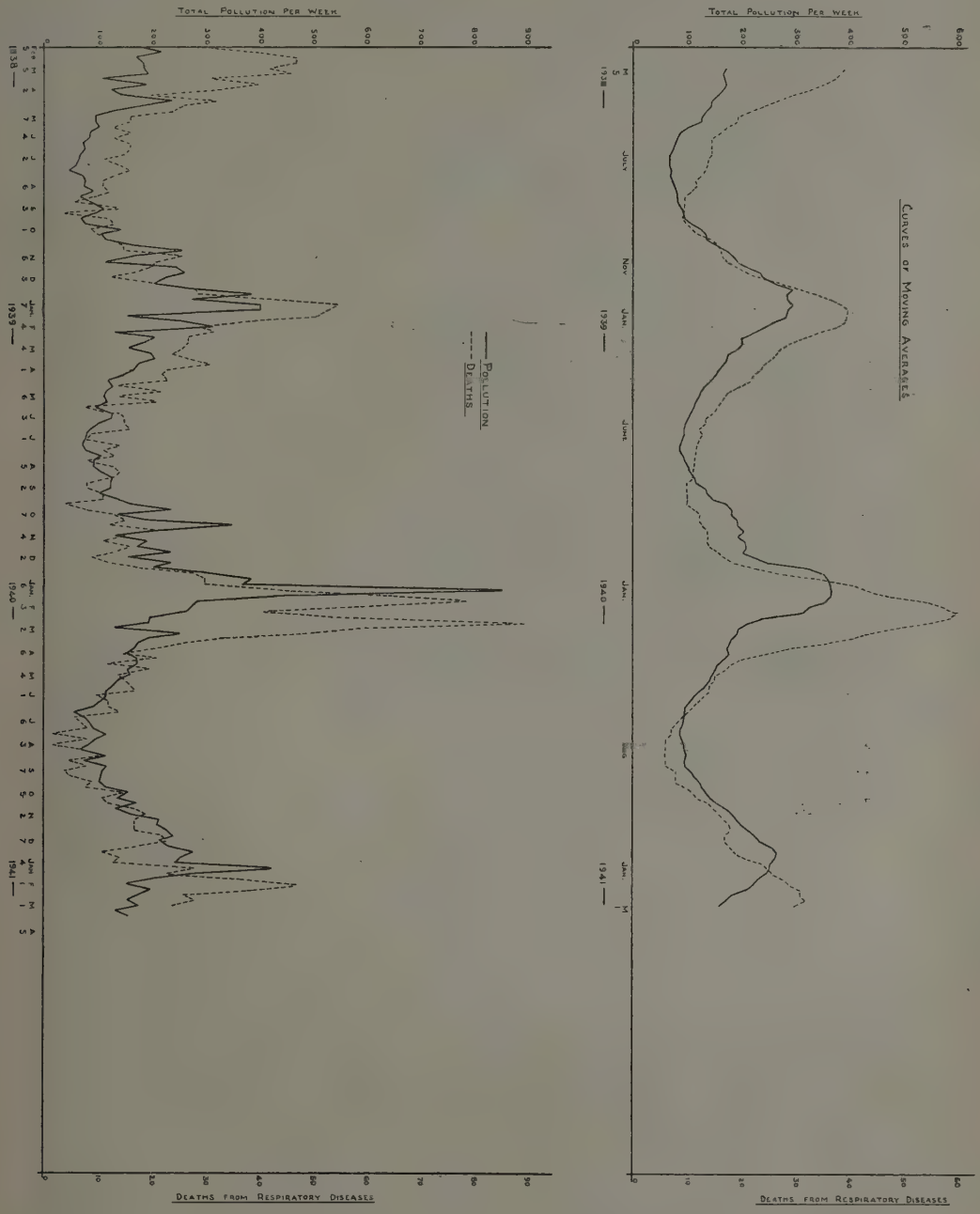

FIG. 2.

Daylight Measurements.

Fig. 1 gives the graphs for daylight measurements by the potassium iodide method at Merrion Street and the Albert Farm. The results are expressed as the daily average of iodine liberated during each week, thereby showing clearly the persistent loss occurring in the city.

The top graph gives the loss of light at Merrion Street plotted as a percentage of the light recorded at the Albert Farm for the same period.

The maximum daily loss of light occurred in the period January 14th to 15th, being 77%.

Pollution by Suspended Matter and Deaths from Respiratory Diseases.

At Dr. Roy Geary's suggestion we have plotted curves of "moving averages" for both these quantities. The points for the graphs are obtained by summing nine consecutive determinations and taking the average; by deducting the first of the determinations, adding the next (the tenth), and dividing by nine a second point is obtained, and so on.

Fig. 2 shows the graphs of moving averages on top with the curves of weekly totals below. The moving average curves smooth out the peaks in the lower graphs very satisfactorily and apparently one should be able to assess the period of lag between the maxima of pollution and deaths. In 1939 the lag was three weeks, in 1940, four weeks, and in 1941 eight weeks, so that it is not feasible to decide on a definite time lag from the data so far available.

The authors wish to express their thanks to Mr. M. G. Dowling for copies of the weekly returns of births and deaths, and to University College for continuation of a grant in aid of the work.

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No. 42.

ESTIMATION OF AMMONIA AND UREA BY A MODIFICATION OF THE CONWAY DIFFUSION METHOD.

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Trinity College, Dublin.

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In all the experimental work on ureagenesis in the liver, and on kidney efficiency in the healthy and diseased, a large number of urea and ammonia determinations have been the main part of the method of approach. From time to time, therefore, the methods generally used for these determinations have been criticised and modified. The most thorough review and test of this description, which takes account of the diffusion method, is the work by Lee and Widdowson (1). These workers come to the conclusion that from all the current methods tried out, including their own modification, Barrett's method (Nesslerization) is the only one suitable for clinical work. Conway's diffusion method is excluded on the grounds of its specialised technique and apparatus and also for its relatively low range. This opinion has been voiced by other workers as well.

In clinical work the diffusion method of analysis is most desirable, and it is astonishing that so far only one modification of the original method has been suggested, which is also a simplification. As originally described by Abelin (2), this modified method exhibited serious difficulties in the end-point titration. The work submitted here deals with a simple modification, which, in the author's opinion, should make Abelin's method an extremely useful one, especially in clinical research.

Abelin's Method.

In this method the Conway Units are cleansed and prepared in the same manner as described by Conway. The inner compartment is then filled with 1 ml. of 2% boric acid which has just been boiled and is still warm. This boric acid receives one drop of indicator, and the unit is ready for ammonia

absorption. At the end of the period of absorption, the ammonium borate formed is directly titrated with N/100 H_2SO_4 or N/100 HCl from a 2 ml. micro-burette.

There is no difficulty in the preparation of any of these reagents, and the only reagent that must be absolutely quantitatively correct is the N/100 acid; the boric acid need only be approximately 2%, since excess is all that is required. This concentration of boric acid is capable of absorbing a great deal more ammonia than the inner compartment of the unit is capable of holding in equivalent of titration acid. Difficulties will, however, be experienced with the use of Abelin's indicator, which is a Tashiro indicator prepared in the following manner:—Make up a 0·1% alcoholic methyl red and 0·1% alcoholic methylene blue, and mix 100 ml. of the former with 25 ml. of the latter (4 : 1). Take one volume of this solution and dilute it with one volume of alcohol and 2 vol. of CO_2-free water. Add drop by drop N/100 NaOH to this solution until the red colour is just discharged. This preparation is diluted 1 : 9 with CO_2-free water, and one drop of it is added to the boric acid in each unit. Abelin claims that this indicator is green at a pH of 5·6, and red at a pH of 5·3–5·2. 2% boric acid has a pH of 5·2–5·3, and, therefore, is red with the indicator when the unit is set up, changes to green the moment ammonia begins to be absorbed, and is titrated back to red. The results published by Abelin show that duplicate estimations have a negligible titration difference, and that ammonia recovery from standard solutions is for all practical purposes 100%. These results so far have not been confirmed.

Conway, who gave this method a trial in his laboratory, makes the following criticism in his book, "Micro-Diffusion Analysis and Volumetric Error" (3):— "The author has subsequently investigated the method, but considers the end-point unsatisfactory when compared with the barium hydroxide titration." Estimations carried out in this laboratory fully support this view, and to some extent Abelin must have realised the uncertainty of his end-point, because he advises continuing the titration until the colour matches the original colour, which has been preserved in an unchanged blank unit. This, however, is a most unsatisfactory procedure; because once the titration has changed from the green to the faintest red, more acid will only increase proportionally the depth of tint until the colour has arrived at the point where further addition will make no difference to the intensity of red. This makes over-titration easy. The end-point should be decided by colour shade and not by colour intensity.

Abelin's unit when set up contains, roughly speaking (reckoning 20 drops to 1 ml.), 1/80th the amount of Tashiro indicator that the Conway unit contains. The colour produced is so faint that only with prolonged experience can the end-point changes be clearly made out.

These are technical difficulties only, and Conway's comment on the method may rightly be quoted at this point: "The principle is certainly a good one, and if in practice not found particularly good in its present form, may very well prove advantageous by an alteration in the conditions or the reagents."

Alterations in Conditions and Reagents.

All alterations were adopted only after a long period of trial, when they had definitely proved themselves simplifications. No loss of accuracy was incurred by adopting these simplifications, they concern:—(*a*) the indicator, (*b*) the seal, (*c*) the urease preparation.

The Indicator.—It is true that Conway's hydrochloric acid-barium hydroxide titration shows an exceedingly sharp end-point with Tashiro's indicator. Such a sharp end-point can hardly be expected when one is titrating boric acid which contains ammonium borate with N/100 HCl, since the reactions take place in a well-buffered medium, of which the change in pH is a gradual one. The first step taken was to bring the strength of Abelin's indicator up to the strength of Conway's. This indicator demonstrated well the buffered nature of the reaction when one nears the end-point in the titration. The green fades and changes to yellow, the yellow gradually turns orange, and the solution finally is a fairly intense pink. It is obviously difficult to fix an end-point in such a type of colour-change. The next step was to alter the composition of Tashiro's indicator. This was done with a view to increasing the methylene blue screen in order that it might cover the early yellow change of the methyl red. The indicator thus obtained proved to be a double-change indicator: (alkali)-green—blue—cherry red (acid). The indicator was tested in a known pH range of a buffered solution (B.D.H. buffer, according to Prideaux and Ward), and it was shown that the blue phase of the indicator covers the pH range 5·23–5·47. Titrating with this indicator the following colour changes are encountered near the end-point:—The green fades, a drop of acid makes the solution flush deep red, but under stirring (which is absolutely essential) it reverts to green, then becomes almost colourless, and this on closer inspection actually is the blue phase, which then needs either a fraction of a drop or a few drops more to establish an easily recognised permanent pink, which no amount of stirring can get rid of—this is the end-point. These changes are easily recognised if one observes the wall of the inner compartment obliquely instead of vertically. This is done by mounting the unit on a relatively high bench nearly at eye-level of the operator. Thus, delicate colour changes at the periphery of the meniscus can be detected.

The Seal.—Vaseline and a mixture of vaseline and hard paraffin have commonly been employed. In practice both these fixatives make the fingers of the operator greasy. Where incubation is desired, no substitute was found for the vaseline-paraffin mixture (3 : 1). In routine clinical work, however, it should be of advantage not to incubate the units, and in that case the following measures will be found adequate and satisfactory:—Place half the number of the lids used in the experiment on a level table with the ground side up, and put 5–6 drops of liquid paraffin in the centre of each slide, using an ordinary dropping pipette; now, turn the remaining half of the lids on top of the ones

thus prepared. A very fine film of paraffin will spread between the ground surfaces of the two lids. The two lids are slid apart when the units are ready to be covered. If the correct amount of paraffin is used the lids will not tend to slide. This method offers three advantages:—Firstly, the operator's fingers remain free of grease during the whole of the experiment; secondly, the fine film of paraffin has rendered the slide perfectly translucent, any change in the reagents can be easily observed; and thirdly, the cleaning of the slides and units is facilitated.

The Urease Extract.—There are two minor practical disadvantages in Conway's preparation of the Urease extract. The glycerol-urease takes a long time to filter (48–72 hrs.) and whenever the filtrate is required, it has to be diluted with phosphate buffer and water. A small variation in the method of preparation produces an extract of equally high qualities, which filters rapidly, and is kept ready for use. Transfer 5 g. of Jack bean meal into an Erlenmeyer flask, add 10 ml. of distilled water, and agitate vigorously for at least 20 minutes, then add 40 ml. of glycerol and 50 ml. of a phosphate buffer of pH 7·4 (3 g. anhyd. Na_2HPO_4 + 2 g. anhyd. KH_2PO_4 in 100 ml. H_2O). Shake this mixture well, allow to stand for a minute, and decant the fluid on to a large filter paper. At a temperature of about 20–23° C. filtration will be complete within a short time. Put the filtrate into the ice-box immediately. It can be kept conveniently in a bottle carrying a graduated 1·0 ml. pipette. This extract is ready for use; 0·2 ml. can be run in succession from the pipette into each unit, or a dropping pipette may be used once it has been calibrated. The extract keeps several months.

Suggested Routine Method.

The method here described should be suitable for clinical routine work. The following reagents are required:—

(1) 2% pure boric acid (*Analar*) with incorporated indicator. This is prepared in the following way:—Pipette 0·5 ml. 0·1% alcoholic methyl red and 0·5 ml. 0·1% alcoholic methylene blue into a flask, and add to it 60 ml. of recently distilled water and 20 ml. of alcohol. Now add drop by drop very dilute alkali (N/500 NaOH) until all the red has been discharged and the solution has assumed an olive green colour. 2 g. of boric acid is now added, and the flask is held in a hot-water bath until all the boric acid has dissolved. If the distilled water has not been recently boiled, it is of advantage to bring the solution to a quick boil. (Boiling does not affect the shade or stability of the indicator.) After the flask has cooled, the solution is made up to 100 ml. in a volumetric flask. This solution is of purple colour; the indicator is of the same concentration as that used in the Conway acid.

(2) N/100 HCl.—In making up this acid from an accurate N/10 standard acid it may be of advantage to incorporate some indicator, as a coloured

solution makes the reading of the meniscus in the micro-burette easier, and the concentration of the indicator in the unit is kept the same throughout the titration. To do this proceed as follows:—Measure 1·25 ml. of 0·1% alcoholic methyl red and the same amount of alcoholic methylene blue into a 250 ml. volumetric flask, to this add 50 ml. of alcohol and 150 ml. distilled water, then proceed to bring the solution to the neutral point with dilute alkali, as described for the boric acid under (1). To this neutral solution 25 ml. of the N/10 acid is added, and the flask is filled to the mark with distilled water.

(3) Urease Extract.—The preparation as described.

(4) Saturated Potassium Carbonate.—Dissolve 112 g. in 100 ml. of boiling water, filter while hot. The cold solution should contain some undissolved salt, otherwise add more of the salt.

(5) Liquid Paraffin.—Used as described in the previous section.

The units, which were thoroughly cleaned with running hot water and soap after their last use, are stored in dilute acid, to which some indicator has been added. The first step, therefore, is to take the units required out of the acid and rinse them with running distilled water, they are then inverted on a clean surface such as a filter paper. While the water is thus allowed to run off for some minutes, the lids are prepared with the liquid paraffin as described above. The units are now filled; they need not be completely dry. From a 5·0 ml. graduated pipette run in succession 0·5 ml. of distilled water into each outer compartment. Take a 1·0 ml. pipette graduated to the end of the tip, and run 1·0 ml. of boric acid into each inner compartment; do not blow out the pipette. For one blood urea estimation three units are needed, in two of which is estimated all the ammonia not produced by the hydrolysis of urea, such as the preformed ammonia contained in the distilled water and urease preparation, which is estimated in one blank unit, and the preformed blood ammonia which is estimated in the second blank unit; in the third unit the urea-ammonia is estimated, as well as all the extra-ammonia just mentioned. According to this plan, the outer compartments of the three units receive in addition to the distilled water in order:—"Blank one," 0·2 ml. of the urease preparation; "blank two," 0·2 ml. of the blood; "unit three," 0·2 ml. blood and 0·2 ml. urease preparation. Each unit is immediately closed with the lid. (*Note*: the blood is added before the urease, and the blood pipette *only* is rinsed twice with the distilled water in the outer compartment.) The units are left for at least 20 minutes on the bench to allow for the urease action in unit three; however, no harm results if they are left a little longer. After this time all three units receive 1·0 ml. of the saturated solution of potassium carbonate; this is run into the outer compartment from a 1·0 ml. pipette graduated to the tip. The outer compartment must be uncovered as little as possible for this purpose, and the pipette must not be blown out. The units remain on the bench for 1½ hours at least; titration can be started whenever convenient after this time. The units may even be left on the bench over night and titrated the next morning. The titration is carried out with the N/100

acid from a micro-burette (2·0 ml.). It is absolutely necessary to stir the fluid in the inner compartment with a fine glass rod during titration; the end-point is reached with the first permanent pink in the solution. It is advisable, at least at the beginning, to do all estimations in duplicate. Every ml. of N/100 titration acid corresponds to 0·3 mg. urea or to 0·17 mg. ammonia. The mg. per 100 ml. of original fluid can easily be calculated; for example, using 0·2 ml. blood in the outer compartment—

ml. titration acid $\times$ 0·3 $\times$ 5 $\times$ 100 = mg. urea per 100 ml. of original fluid.

It will be seen from above that the smallest division of the burette (= 0·01 ml. acid) corresponds to 3γ urea (= 0·003 mg. urea).

"Blank one" (urease ammonia) can be relied upon to be quite negative for the first month at least, after that time a small blank value may be obtained, and the urease solution should not be kept longer than three months. "Blank two" may be omitted altogether for routine clinical work, since blood even after standing for 24 hours has a preformed ammonia value of not more than 1 mg. N/100 ml. This has been pointed out by Conway (Ref. 3, p. 101) and agrees with the present author's experience.

Range and Accuracy of the Method.

The range of the method was investigated first. A 2 "gammil" NH_3–N control solution as recommended by Conway was prepared in order to test the lower limit of the range. This solution is made up as follows:—0·471 g. of analytically pure $(NH_4)_2SO_4$ is dissolved and made up to a litre with distilled water. 2 ml. of this stock is diluted to 100 ml. By making a gradually increasing range of NH_3–N solutions from the stock, the minimum amount of NH_3–N was found which is necessary to change the indicator, or, in other words, the least amount of ammonia which on absorption by the boric acid will shift the pH from 5·23–5·47. This amount was found to correspond to 10γ NH_3–N, and approximately equals the amount of ammonia formed from 0·2 ml. of a solution of 4 mg. % urea. One simple modification would allow one to estimate even more dilute solutions of urea, i.e. the use of 1·0 ml. of analytical fluid in the outer compartment instead of 0·2 ml. This would allow the estimation of 1·0 mg. % urea and slightly less. Incidentally, since 1·0 ml. is the ideal quantity for the outer compartment of the Conway unit, it should be used when obtainable. The upper limit of the range was not sought; 2% boric acid is capable of absorbing a great deal more ammonia than it is possible to titrate with a N/100 acid (HCl) in the inner compartment of the Conway unit. The inner compartment easily will hold 3·0 ml. of titrating acid in addition to the 1·0 ml. of boric acid, always remembering that thorough stirring has to be employed. With some experience, even 4·0 ml. can be safely used. Taking 3·0 ml. as the maximum, the highest level for blood urea (using 0·2 ml.) would be 450 mg. %. This covers the whole range of clinical blood urea values, and no dilution of analytical fluid is needed.

The accuracy of the method was investigated next. For this purpose the routine method of the technique described was employed simultaneously with the original Conway method. 600 mg. of pure urea (Kahlbaum) were dissolved and made up to one litre with distilled water, and a few drops of toluol were added to preserve it. From this stock various dilutions were prepared. Simultaneous duplicate estimations were carried out on these dilutions with both methods. Conway's Range II solutions were employed, i.e. N/200 HCl for the ammonia absorption and N/150 $Ba(OH)_2$ for the titration. The results are given in Table 1. The barium hydroxide titration was done with the standard horizontal burette; in the end-point titration often the fraction of a drop was used. The boric acid was titrated from a 2·0 ml. micro-burette, and no attempt was made to deal with fractions of drops when nearing the end-point of the titration. One drop from the burette when accurately delivered measures 0·01 ml. The reading on the burette was always taken to the nearest 0·01 ml. division. It is therefore not surprising to find the same error throughout the whole range of dilutions, i.e. an error of $\pm 3\gamma$ or at the most $\pm 6\gamma$ which corresponds to $\pm$ 0·01–0·02 ml. of titration acid. It is for this reason that the results, expressed as percentage recovery of added urea, do not clearly reflect the true state of affairs. When doing a large number of estimations over a long period of time, it will be found that by far the most frequent error is $\pm$ 0·01 ml. acid. This will be seen from a representative sample of titrations carried out largely on clinical material or on tissue juices, the figures of which are given in Table 2.

TABLE 1.

γ Urea added.	γ Urea recovered by Conway's method.		γ Urea recovered by the Boric acid method.		
	Unit I.	Unit II.	Unit I.	Unit II.	% (aver.).
12	11·60	11·58	15	9	100·00
24	23·00	23·40	21	18	81·25
48	47·20	46·00	48	45	97·00
72	70·80	70·20	72	66	95·83
96	94·60	95·20	96	96	100·00
120	119·20	118·60	117	117	97·50
240			240	243	100·62
360			360	363	100·41
480			480	474	99·35
600			597	600	99·75

TABLE 2.

	ml . N / 100 HCl.								
	1	2	3	4	5	6	7	8	9
Unit I. ..	0·36	1·69	0·48	0·48	0·28	0·62	0·31	0·33	0·46
Unit II. ..	0·34	1·72	0·50	0·48	0·27	0·62	0·31	0·34	0·46
Unit III. ..	—	—	—	—	—	—	—	0·33	0·44

It should be possible with the boric method to obtain results as shown in Table 1 quite easily in clinical work. The figures show a standard deviation of 1·5 mg./100 ml. for 0·2 ml. samples, or 0·3 mg./100 ml. for 1·0 ml. samples. Conway in his work (4) shows an accuracy of 0·8 and of 0·52. The error involved as already pointed out is chiefly one of titration, and as the method of titration is refined, the error is decreased. Three steps can be taken in this direction :—one can choose a N/150 HCl instead of N/100, one can deal with fractions of drops near the end-point delivery, and one then can read the meniscus to the nearest half 1/100th division of the 2·0 ml. micro-burette. Duplicate estimations performed under these circumstances are given in Table 3. In order to obtain accurate readings to the 0·001 ml. of titration acid it would of course be simplest to use the N/150 acid in the horizontal burette. The error expressed as standard deviation under these circumstances need not be greater than 0·005 ml. titration acid or 0·5 mg. % if 0·2 ml. of fluid is analysed. Table 3 illustrates this sufficiently. The accuracy of the method compares favourably with Conway's method.

TABLE 3.

	ml . N / 150 HCl.				
	1	2	3	4	5
Unit I	0·030	0·095	0·170	0·260	0·340
Unit II	0·025	0·100	0·175	0·260	0·340

The advantages of the boric acid method over the original Conway method are many. No special piece of apparatus is required except for the diffusion unit, no alkali-acid factor enters into the calculations, and no dilution of the clinical material is required, since the range covers all possible clinical values. The method should not be employed for urea values below a concentration of 5 mg./100 ml. unless 1·0 ml. of analytical fluid is put into the outer compart-

ment, when one might go as low as 1 mg. %. This drawback certainly is not a serious one for clinical purposes. The direct titration employed in the boric acid method also makes it possible to use two different strengths of acid in the same titration. An increased accuracy is obtained with the N/150 acid when dealing with low values, so it is advisable to start off using this strength of acid. If after having added 2·0 ml. of this acid, the end-point is not reached, one can change over to and continue the titration with the N/100 acid until the end-point is reached. This involves no complicated calculation, since the equivalent amount of urea to every ml. of either acid is exactly known. This is the procedure of choice for research in which urea values between 4 and 400 mg./100 ml. are met with.

SUMMARY.

(1) Abelin's method for the diffusion analysis of ammonia and urea has been investigated.

(2) The method has been found unsuitable in its original form. Certain modifications were tested over a period of 2 years, and a method suitable for clinical routine work as well as for research purposes has been described.

(3) The range of the method lies between 4–400 mg. urea per 100 ml. The recovery of urea by this method has been shown to be quantitative. Under ordinary working conditions with a 2·0 ml. micro-burette the error is ± 1·5 mg. urea/100 ml.; certain refinements will reduce this error to ± 0·5.

(4) The method compares favourably with the original Conway method against which it has been tested.

In conclusion the author wishes to take the opportunity to thank Professors W. R. Fearon and E. J. Conway for the helpful criticism and advice they have given in this work.

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No. 43.

THE PHENOL AMIDINE REACTION: THE DETECTION OF GUANIDINE, GUANIDINE DERIVATIVES, AND UREA, BY MEANS OF THYMOL AND HYPOCHLORITE.

By WILLIAM ROBERT FEARON.

[Read April 22. Published separately July 28, 1941.]

In 1925, Sakaguchi described a reaction characteristic of the monosubstituted guanidines, in which red pigments are obtained when α-naphthol and the guanidine compound are treated with hypochlorite in strongly alkaline solution. Positive results were obtained with arginine, guanidino-acetic acid (glycocyamine), and guanidino-butyric acid; while free guanidine, nitroguanidine, nitroarginine, glycocyamidine, creatine, creatinine, and various urea derivatives yielded no pigments. From this, Sakaguchi concludes (1925, 1) that the test is dependent on the presence of the system $R.NH\text{–}C(NH)\text{–}NH_2$. Proteins give a positive reaction owing to their arginine content, and the test, suitably modified, has become of value in the estimation of arginine in protein hydrolysis mixtures.

Although Sakaguchi isolated and analysed one of the pigments obtained, he does not appear to have investigated the mechanism and limiting conditions of his test. An early examination made by the present author showed that the α-naphthol could be replaced by other common phenols, provided that they were unsubstituted in the *para* position, and the scope of the reaction has been surveyed by Poller (1927), who reports positive results with dicyandiamide, methylguanidine, and symmetrical dimethylguanidine; and negative results with urea and asymmetrical trimethylguanidine, thus confirming Sakaguchi's conclusions as to the specificity of the reaction.

Recently, in testing for arginine in liver protein extracts, it was observed that the Sakaguchi reaction was untrustworthy, and gave a deep purple colour that could not be matched with the carmine colour given by arginine or arginine-containing proteins, such as caseinogen and gelatin. The purple colour obtained in the liver extract was found to be due to contamination by iron porphyrin, probably haem, which is able to catalyse the oxidation of α-napththol by hypochlorite in strongly alkaline solution. A similar effect with haemoglobin has been noted by Sakaguchi, and made the basis of a delicate test for blood (1925, 2).

With the object of avoiding this extraneous colour production, a re-examination was made of the effect of substituting other and less reactive phenols for the α-naphthol. Out of a number of common phenols examined,

thymol was found to be the most suitable, and by controlling the alkalinity of the medium, the range of the test has been extended so as to include both guanidine and urea, neither of which yields pigments in Sakaguchi's original form of the reaction.

The Thymol Amidine Reaction.

Principle: Compounds containing the amidine group when treated with a minimum amount of hypochlorite in alkaline solution couple with phenols unsubstituted in the *para* position to yield quinonoid pigments. In weakly alkaline solutions (pH 8·5–pH 10), urea, free guanidine, and monosubstituted guanidines yield golden-yellow pigments with thymol. In strongly alkaline solutions (pH > 11), neither urea nor guanidine reacts, and the test is, in general, characteristic of substituted guanidines of the type R . NH–C(: NH)–NH_2 or R . NH–C(: NR)–NH_2.

Reagents: (1) Thymol in saturated aqueous solution. This contains about 0·3 per cent. of the phenol, and is preferable to stronger solutions in alcohol or other solvents liable to be attacked by the hypochlorite during the course of the test.

(2) Sodium hypochlorite, 2 per cent., representing about 1 per cent. available chlorine.

(3) Sodium carbonate, 2 per cent.

(4) Sodium hydroxide, 20 per cent.

Method: A. *Guanidine Derivatives.*—About 3 ml. of the solution is made strongly alkaline with a few drops of 20 per cent. sodium hydroxide; 3–6 drops of the hypochlorite are added, followed by about 1 ml. of the thymol solution. If the test be positive, a golden-yellow colour develops and persists for several hours.

The test is positive with methylguanidine and other monosubstituted guanidines, including guanidino-acetic acid and arginine, both free and in protein form. It is also given, though more slowly, by asymmetrical dimethylguanidine. The test is negative with symmetrical triphenyl guanidine, creatine, creatinine, dicyanamide, and, under the specified conditions of alkalinity, with guanidine salts and urea, and with all mono- and disubstituted ureas examined.

Although, as might be expected, the thymol reaction is not as rapid or as sensitive as the α-naphthol reaction, it is capable of detecting 0·1 mg. of arginine carbonate in 2 ml. of water. In absence of excess of hypochlorite, the pigments are stable, and, with the exception of those obtained from unhydrolysed proteins, are readily extracted by amyl alcohol.

B. *Guanidine and Urea.*—The guanidine solution, previously neutralised if necessary, is treated with an equal volume of a boric-phosphate buffer of pH 9–pH 10, or with a few drops of 2 per cent. sodium carbonate. A couple of drops of the hypochlorite solution are added. Excess must be avoided, otherwise an orange colour is given by free guanidine in absence of any

phenol. This is the "guanidine-hypochlorite reaction," and will be discussed subsequently. After addition of the hypochlorite and 2 ml. of the thymol solution, on mixing, a yellow colour develops similar to that given by the substituted guanidines, and also extractable by amyl alcohol.

The test for urea is carried out in the same way. Both urea and semicarbazide yield pigments similar to the guanidine pigments, but tending to turn green if the hypochlorite and phenol be in excess, owing to secondary formation of an indophenol pigment.

None of the other monosubstituted and disubstituted ureas examined gave the test.

MECHANISM OF THE REACTIONS.

From an inspection of the formulae of the reacting compounds, the test reveals the presence of the free amidine group $-C(NH)-NH_2$, such as occurs, potentially at least, in guanidine base, urea, semicarbazide, the monosubstituted guanidines, and symmetrical dimethylguanidine. The negative result obtained with creatine offers additional support for questioning the conventional structural formula for creatine, the anomalies of which have been pointed out by Hunter (1928).

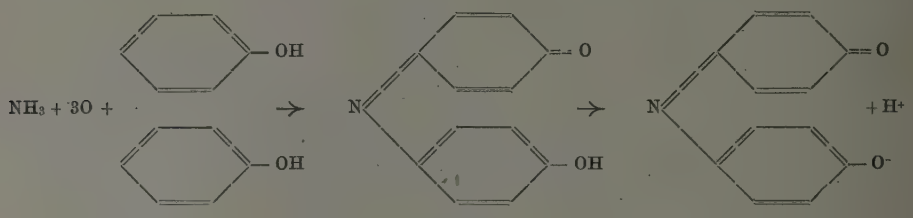
Although the pigments can be separated from the reaction mixtures by means of amyl alcohol, up to the present it has not been possible to obtain them in crystalline form suitable for analysis. Large-scale methods of preparation from the two most readily available sources, namely, guanidine carbonate and urea, have yielded products contaminated with indophenols and thymol oxidation derivatives that are hard to separate. As the indophenol reaction is liable to occur whenever ammonia is generated in presence of a phenol and a hypohalogenite, its conditions and mechanism must be considered.

The Indophenol Reaction.—The reaction appears to have been observed first by Berthelot (1859), who noticed the formation of blue pigments when amino compounds are treated with phenol and hypochlorite. In 1873, Fluckiger described the formation of a similar type of pigment when bromine interacts with phenols in ammoniacal solution. In later years the test has been rediscovered several times (Gibbs, 1926), and it has been adapted successfully for the estimation of ammonia in cerebro-spinal fluid (Thomas, 1912, 1913) and in urine (Orr, 1924). Thymol has been used instead of phenol by Lapin and Hein (1934), and Miller (1935) has employed the reaction as a means of detecting amino acids. In Miller's test, 2 ml. of 0·01 M amino acid solution are treated with 8 drops of 5 per cent. phenol and 2 ml. of 2 per cent. hypochlorite.

A blue colour gradually develops with the common amino acids, the most important exceptions being phenylalanine, tyrosine, tryptophane, histidine, and aspartic acid. Taringi and Lenci (1912) have suggested a mechanism for the general reaction that involves the intermediate formation of a *p*-nitroso phenol,

and its subsequent condensation with another molecule of the phenol to form a phenylimido-quinone.

A simpler explanation appears to be the chlorination of the ammonia, and its subsequent interaction with two molecules of the phenol, one of which is then oxidised to the quinonoid form. The test is given by all ammonium compounds, and by substances, such as urea and guanidine, that liberate ammonia in presence of alkaline hypochlorite. The response obtained with the amino acids in Miller's test can be explained by their relative instability to hypohalogenites, as shown by Werner (1937). The common aliphatic amines do not undergo deamination readily under the conditions of the test, and do not form indophenol pigments.



Indophenol (red) Indophenol ion (blue)

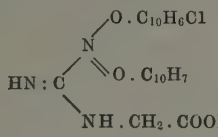
Indophenol formation can be repressed in the phenol amidine test by working with the minimal amounts of hypochlorite and phenol necessary for development of the yellow pigment.

The Phenol Amidine Reaction: (1) *The selective effect of the alkali.*—The degree of alkalinity of the reaction mixture affects both the phenol and the hypochlorite, and, possibly, the configuration of the compound attacked. In solutions more acid than pH 8, both α-naphthol and thymol are oxidised, and may be chlorinated by hypochlorite. α-naphthol yields purple precipitates of complex nature; thymol forms an opalescent precipitate of thymoquinone that turns red on addition of alkali. In weak alkaline solutions of pH 8·5–pH 10, α-naphthol is similarly attacked by hypochlorite, while thymol is much more slowly affected, unless the reagents are in excess. In stronger alkaline solutions of pH > 11, neither the α-naphthol nor the thymol reagent is visibly affected. Hence both reagents may be used for the detection of substituted guanidines in strongly alkaline solution, whereas the α-naphthol cannot be applied satisfactorily for the detection of urea and guanidine, both of which only react in solutions of low alkalinity.

The explanation of this effect of the alkali may be due to the fact that hypochlorous acid is a weak acid, and exists to some extent in the un-ionised form in weakly alkaline solutions, while in solutions more alkaline than pH 11, it is converted into the ionic form ClO^-, which is less effective as an oxidising and chlorinating agent than the un-ionised acid. The amidine group of both urea and guanidine appears to exist in some masked form relatively resistant to the

chlorinating action of ClO^-, but vulnerable to HClO, hence the pH of the mixture determines within a fairly narrow range the scope of the reaction. Excess of concentrated alkaline hypochlorite destroys both urea and guanidine (Fenton, 1879), but these conditions do not occur in the amidine test.

(2) *Constitution of the Pigments.*—From 30 gm. of guanidino-acetic acid, Sakaguchi has obtained 2 gm. of the α-naphthol pigment, $C_{23}H_{18}N_3O_4Cl$, which he regards as having the structure:



This formulation, however, does not offer an explanation of the intense colour of the compound, or the fact that it can be bleached by careful reduction with hydrosulphite, and re-oxidised back to the original shade in the manner characteristic of the quinonoids. The colour and general properties of the pigments obtained with thymol, phenol, and other monocyclic reactants closely resemble those of a phenylimido-quinone of the type $O : C_6H_4 : N . C_6H_5$, and it is suggested that the pigments obtained in the reaction are of an imidino-quinone type, in which the two aromatic rings are united to different nitrogen atoms. This suggestion is supported by four observations:

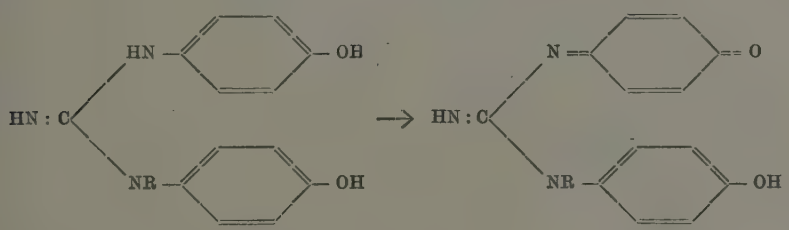
(1) Symmetrical dimethyl guanidine at pH 9–pH 10 reacts with thymol and hypochlorite to yield the characteristic colour. Here, the configuration of the compound does not allow of two phenol groups being coupled to the same nitrogen atom as in Sakaguchi's formula.

(2) Symmetrical diphenyl guanidine at pH > 11, on addition of hypochlorite, in the absence of thymol yields a characteristic yellow colour. If the solution is not sufficiently alkaline, further changes ensue, resulting in a dark brown mixture of pigments.

(3) Substituted phenols in which the *para* position is occupied fail to react, which implies that the coupling in the colour tests involves the *para* position of the reacting phenol.

(4) The test is negative with ammonia, hydrazine, cyanate, aliphatic amines, aliphatic amides, ureides, and all the common amino acids, other than arginine.

Hence it is concluded that the products obtained in the phenol amidine reactions are of the following general type:—



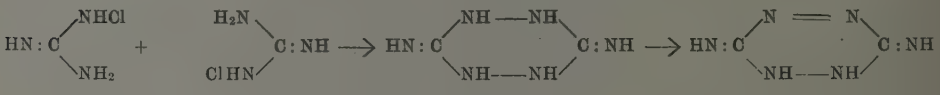
Leuco-form. Pigment.

The yellow colour of the pigments is ascribed to the presence of the imido-quinone system, $O : C_6H_4 : N–R$, which has been discussed by Stieglitz (1924).

These formulae do not take into account the possibility that one or more chlorine atoms may be present as substituents in the benzene rings, although the fact that the pigments once formed are stable in strongly alkaline solution makes it unlikely that the imino hydrogen has been replaced by the halogen.

The Guanidine Hypochlorite Reaction.—As is well known, guanidine salts under appropriate conditions yield an orange colour with hypochlorite solutions, and this reaction may complicate and confuse the phenol tests.

The hypochlorite reaction, like the thymol reaction, works best in the region pH 8·5–pH 10. If the solution be too alkaline, the colour is not obtained, and for this reason the test is negative when dilute hypochlorite is added to a solution of guanidine carbonate, while the test is positive when hypochlorite is added to a solution of guanidine acetate. The guanidine hypochlorite reaction differs from the thymol reaction in that it requires considerably more than two equivalents of hypochlorite, and the pigment is much less soluble in amyl alcohol. A similar reaction is obtained with semi-carbazide and hypobromite, and the pigment has been obtained as an orange, crystalline powder by Lynch (1912), who found it to be a derivative of *p*-urazine. By analogy, the guanidine hypochlorite pigment is either 3 : 6-di-imino-4 : 5-dihydro-1 : 2 : 4 : 5; tetrazine, or a simple chloro derivative.



Pigment.

Neither urea nor any of the substituted guanidines examined yielded a colour with the hypochlorite reagent alone.

Summary.

1. In alkaline solutions of pH 8·5–pH 10, urea, guanidine and mono-substituted guanidines, on treatment with hypochlorite, react with thymol and similar phenols to yield stable yellow pigments of a quinonoid type.

2. In more alkaline solutions of pH > 11, only the substituted guanidines react, and the test becomes a modification of Sakaguchi's *a*-naphthol reaction.

3. The thymol test is suitable for the detection of arginine, both free and combined in protein form.

4. The conditions and mechanism of the indophenol reaction for ammonia and the hypochlorite test for guanidine are discussed.

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No. 44.

THE HYDROLYSIS OF LAMINARIN.

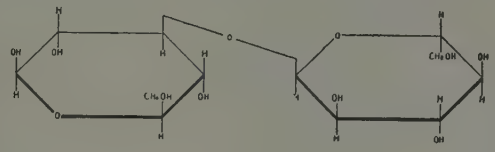
ISOLATION OF A NEW GLUCOSE DISACCHARIDE.

By VINCENT C. BARRY, D.Sc.,

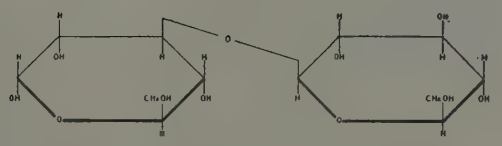
University College, Galway.

[Read May 27. Published separately July 28, 1941.]

Methylated laminarin has been shown to yield on hydrolysis only one trimethyl glucose (1). Unlike the trimethyl glucose resulting from the hydrolysis of methylated starch, cellulose, glycogen, etc., this proved to be 2 : 4 : 6 trimethyl glucopyranose. The existence in laminarin, therefore, of a linkage quite different from that in the other glucose polysaccharides mentioned above, was evident. It was thus suggested (*loc. cit.*) that it should be possible to obtain from it a disaccharide quite distinct from maltose or cellobiose, and having the structure shown in I or II.



I



II LAMINARIBIOSE

It has now been possible to show that a new disaccharide is present among the cleavage products of laminarin when hydrolysis of the latter is catalysed by enzymes or acids. The disaccharide was first isolated in the form of its phenylosazone, which crystallises in a very characteristic form. Finally, by partial acid hydrolysis of laminarin, the disaccharide was isolated in the form of a syrup,[1] or a white amorphous powder, by fractionation of the products of hydrolysis. It is proposed to name this sugar *laminaribiose*. (Laminariose

[1] On one occasion, after long standing in the air, this syrup crystallised to a considerable extent, forming colourless rectangular prisms, having a melting-point 161–162° C.

was the name given by Kylin (2) to a substance with the formula $(C_6H_{10}O_5)_2 . H_2O$, which, according to him, existed in aqueous extracts of laminariae. No evidence has been found in this laboratory to suggest the existence of any free disaccharide in the aqueous extracts of laminariae.)

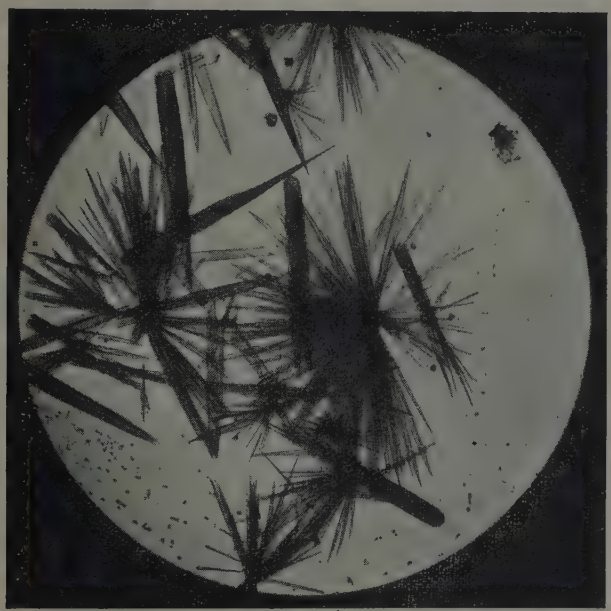
As already pointed out (1), the properties of laminarin and its derivatives suggested that it was composed, like cellulose and lichenin, of β-glucose units. This prediction has been confirmed by a study of the behaviour of oligosaccharide fragments of the laminarin molecule towards enzymes. Colin and Ricard (3) showed that laminarin was fermented by snail juice. That observation has been confirmed, but no other enzyme source has yet been found capable of splitting the polysaccharide molecule. Yeast, ptyalin, emulsin, and diastase, all appear to have no action. With snail juice taken from the crop and stomach of *Helix pomatia* or *Helix aspersa* hydrolysis proceeds rapidly at ordinary temperatures, bringing about the conversion of the polysaccharide to glucose. In the course of the hydrolysis it is possible to show the existence in the liquid of laminaribiose by isolating it in the form of the osazone. It is probable, therefore, that the snail juice contains, among other enzymes, a distinct disaccharidase (laminaribiase), which catalyses the breakdown of laminaribiose to glucose. The action of this enzyme must be very rapid, as at no time during the course of the fermentation was it possible to prepare more than a very small quantity of laminaribiosazone from the hydrolysis mixture.

No systematic method of selective adsorption has so far been tried to bring about the elimination of the disaccharidase from the juice. The juice may be precipitated by alcohol and dialysed without altering the nature of its action on laminarin. At temperatures as high as 75° C. deactivation of the disaccharidase is not effected, and glucose is slowly produced from laminarin. Varying the pH from 4·5 to 9·1 altered the rate of hydrolysis, but glucose still remained the main product of the reaction, although when the pH was high on the alkaline side the breakdown was very slow, and the presence of glucose could only be demonstrated after some weeks. Other sources of a laminarin-splitting enzyme were sought. The sea-urchin is supposed to feed on fragments of sea-weed, and it was accordingly thought that an active ferment would be present in this echinoderm. The brown juice obtained from this source was found, however, to be inactive. A similar juice from the periwinkle (*Littorina litorea*)—also a known feeder on the fronds of laminariae—appears to contain no active ferment.[2]

The hydrolysis of the polysaccharide may be easily effected in the cold with concentrated hydrochloric acid or on the boiling water-bath with dilute mineral acid. If the course of the reaction is followed with the polarimeter, it is possible to interrupt the hydrolysis at different stages. With the different

[2] Since this paper was read, a glycerine extract of the limpet (Patella) was found to be capable of hydrolysing both laminarin and starch to glucose.

acids it was possible, at different stages of the reaction, to prepare the disaccharide osazone from the hydrolysis mixture. With a view to separating the laminaribiose N. oxalic acid was found to be the most easily controlled. The reaction was allowed to proceed until it was about two-thirds complete, so as to reduce as far as possible the proportion of oligosaccharides present in the liquid. After neutralisation with chalk the liquid was then fermented by yeast, thus destroying the glucose. The liquid remaining was optically active, having a small positive rotation, and contained laminaribiose mixed with higher



LAMINARIBIOSAZONE.

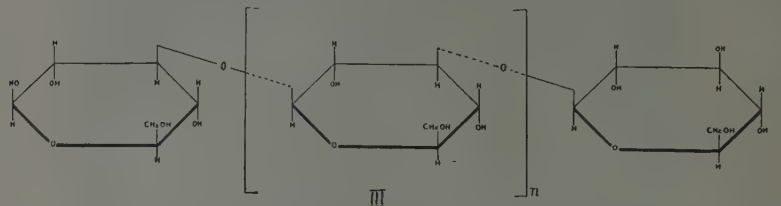
(Magnification = 160 diameters.)

glucose polymers. This mixture, remaining after removal of the water under reduced pressure, was dissolved in methyl alcohol, and fractionated by absolute alcohol and ether. The laminaribiose may be obtained as a white amorphous powder by precipitating with ether from absolute alcohol solution. It has a slightly sweet taste, and yields only glucose on hydrolysis by acid or emulsin. It also forms the beautiful osazone shown in photograph.

If laminarin is two-thirds hydrolysed, as described above, and the oxalic acid then neutralised by chalk, the hydrolysis may now be completed by the addition of emulsin. It is thus clear that the oligosaccharide fragments

resulting from the partial acid hydrolysis of the laminarin are broken down to glucose by the β-glucosidase of emulsin, and so contain β-glucosidic linkages. The laminaribiose itself is rapidly converted to glucose by emulsin, and may accordingly be formulated as glucose-3-β-glucoside (II). If a sufficient quantity of laminaribiose can be accumulated, it will be possible to confirm this structure.

With the isolation of this new glucose disaccharide, and the recognition of the nature of its glucosidic link, it is possible to confirm the structure suggested for laminarin in a previous communication (*loc. cit.*). In III laminarin is formulated as a straight chain of β-glucose molecules linked from the reducing carbon of one hexose to carbon atom 3 of the next hexose molecule.



III

It must, however, be pointed out that from other experiments proceeding in this laboratory it appears improbable that the polysaccharide molecule consists of a simple straight chain.

Zechmeister and Toth (4) found in yeast membrane a glucose polysaccharide with a similar linkage to that occurring in laminarin. They were able, by partial hydrolysis of their substance with strong hydrochloric acid in the cold, to isolate an osazone of a disaccharide. The properties of their osazone are similar to those described here for laminaribiosazone.

Experimental.

Action of Snail Juice on Laminarin.—About 0·5 c.c. of clear brown juice was obtained from the crop and stomach of an edible snail. The alcoholic precipitate from this juice was collected on an immersion glass filter and redissolved in distilled water (2 c.c.). The solution was dialysed for 4 days against distilled water, and then added to a solution of laminarin (1·094 g.) in distilled water (100 c.c.). The rotation of the solution changed from − 0·36° (initial value) to + 1·01° (after 3 days). This amounts to 80 per cent. conversion to glucose. The temperature during the fermentation was kept at 12° C. After 24 hours a fraction of the liquid with the usual treatment gave glucosazone in considerable amount. The filtrate after separation of the glucosazone deposited on slow cooling another osazone, which was recrystallised from boiling water. This was the osazone of laminaribiose described below. The yield, however, was small, and was not improved by taking the hydrolysate

at different stages. When juice from the common snail was used the hydrolysis took a similar course. Measurement of the velocity constant for this enzymatic hydrolysis showed that its value falls off as the action proceeds.

When the action of the snail juice was examined at temperatures between 65° C. and 75° C., the hydrolysis was found to proceed more slowly. At 65° the concentration of snail juice required to produce fermentation was about three times that described above. After 24 hours both glucose and laminaribiose were present in the liquid.

When the pH of the hydrolysis mixture was brought to 9·1 by the addition of the calculated quantities of 0·2 M boric acid in 0·2 M potassium chloride and 0·2 M caustic soda, the presence of glucose could not be shown for 21 days. At pH 8·7 the presence of glucose and laminaribiose was demonstrated in the hydrolysate after 6 days. The pH was brought to the acid side using calculated quantities of 0·1 M acid potassium phthalate and 0·2 M caustic soda. Below pH 4, the action of the enzymes appeared to be inhibited. Above this, both glucose and laminaribiose were present in the liquid.

Acid Hydrolysis:

With Concentrated Hydrochloric Acid.—Concentrated hydrochloric acid (250 c.c.) was added to laminarin (30 g.) in a stoppered flask. The laminarin all dissolved in a few hours, and the course of the hydrolysis was followed with the polarimeter. The rotation in a 1 dcm. tube changed from +1·82° after 20 hours to +5·68° after 120 hours. The final value taken after 18 days was +6·65°. When the reading was +3·95° (48 hrs.), a portion of the liquid was neutralised with barium carbonate, and baker's yeast added to the diluted filtrate. After 48 hours no glucose remained, but the liquid had a small positive rotation (+0·26° in a 2 dcm. tube). The liquid was allowed to stand with chalk for some hours, and the yeast cells were then easily removed by filtration. The filtrate was taken to dryness and extracted with hot methyl alcohol. This extract was treated with absolute alcohol until no further precipitate formed after cooling for 24 hours in the refrigerator. The precipitated material was dried in the desiccator to a white powder, which strongly reduced Fehling's solution, but had no sweet taste. It formed an amorphous osazone soluble in hot water, and was probably a mixture of higher glucose polymers. The alcoholic filtrate was taken to dryness and re-extracted with boiling absolute alcohol. Evaporation of the alcohol gave a yellow syrup which had a sweet taste and a low positive rotation, and yielded laminaribiosazone with the usual treatment.

With N Oxalic Acid.—When laminarin is heated with N oxalic acid solution on the boiling water bath, there is no further change in the rotation after 16 hours. Laminarin (2·5 g.) after 7 hours' heating gave 0·5 g. of crude laminaribiosazone after the usual treatment. The hydrolysis was approximately two-thirds complete at this stage. Interruption of the hydrolysis earlier or

later than this seemed to give a smaller yield of disaccharide osazone. Laminarin (32 g.) was heated with N oxalic acid solution (900 c.c.) as described above. After 7 hours the solution (Rotation + 2·87°) was neutralised with chalk, and yeast added to the neutral filtrate. The rotation of the solution had dropped to + 0·17° after 24 hours, and no glucosazone was got with the usual treatment. The liquid was taken to dryness, leaving a light brown syrup. This was redissolved in distilled water (35 c.c.), and 5 c.c. of the solution after decolorising with charcoal were heated on the water-bath with phenylhydrazine acetate. The burner was withdrawn after ½ hour and a yellow osazone separated slowly. This was washed with water, and was then recrystallised from boiling water with slow cooling. The crystals appeared under the microscope shaped like sword-blades, occurring separately and in rosettes. After two further recrystallisations the crystals melted at 195° C. $[\alpha]_D^{19°C.} = -79·6°$ in alcohol. Found N = 10·73 per cent.; calculated 10·77 per cent. The osazone is easily soluble in hot water, alcohol, and in hot acetone. The disaccharide osazone of Zechmeister and Toth (4) had a M.P. 198° C. and $[\alpha]_D^{20°C.} = -75·3°$ in alcohol.

The remainder of the aqueous solution (30 c.c.) above was evaporated to dryness and extracted with boiling methyl alcohol (200 c.c.). This was reduced to half bulk and absolute alcohol added, until no further precipitate was formed (125 c.c.). After 24 hours in the refrigerator, the precipitated material (*a*) was separated by filtration, and the filtrate again taken to dryness. The residue was extracted several times with boiling absolute alcohol, and the combined extracts (125 c.c.) allowed to stand overnight in the refrigerator. A white powdery material (*b*) separated, which was collected and dried. On addition of excess ether to the alcoholic filtrate a white solid appeared, which was filtered on a glass filter, washed with alcohol and ether, and dried in the desiccator to a white powder (*c*). The alcoholic-ether filtrate left a syrup (*d*), when evaporated under reduced pressure.

Properties of these Fractions.

(*a*) This was obtained after drying in the desiccator in the form of a light white tasteless powder easily soluble in water and methyl alcohol. It reduces Fehling's solution, and is easily hydrolysed by emulsin or by warming with dilute acid to glucose. Its aqueous solution does not give any laminaribiosazone after the usual treatment. It has $[\alpha]_D^{15°C.} = + 0·3°$ (water), and is thus nearly optically inactive. It is probably composed of laminaritriose mixed with higher polymers.

(*b*) A light white deliquescent powder with a slightly sweet taste. It dissolves easily in cold water and less easily in hot absolute alcohol, from which it separates again on cooling. Its aqueous solution yields some laminaribiosazone accompanied by some less crystalline material. It is easily hydrolysed by dilute acid or by emulsin to glucose. It has $[\alpha]_D^{15°C.} = + 15·12°$ (water) 20 minutes

after solution, falling in 24 hours to + 13·5°. This fraction weighed about 1 g., and consists of laminaribiose contaminated by oligosaccharide material.

(*c*) This fraction (1 g.) resembles the previous fraction in appearance and properties. Its aqueous solution with the usual treatment gives without recrystallisation beautiful crystals of laminaribiosazone. Hydrolysis with dilute acid or emulsin gives only glucose. This fraction has $[\alpha]_D^{15°C.}$ (water) = + 20·8° 25 minutes after solution, falling after 21 hours to + 16·14°. The powder softens about 90°, and melts slowly with decomposition to form a milky liquid. The aqueous solution of this fraction on evaporation yields a colourless syrup having a sweet taste. It reduces Fehling's solution, but not Barfoed's solution. This syrup showed no tendency to crystallise.

(*d*) This syrup was small in quantity, and appears to contain some disaccharide. It had $[\alpha]_D^{15°C.}$ = + 5·11° → 3·75° after 24 hours in aqueous solution. Hydrolysis yielded a liquid from which glucosazone was prepared in quantity.

The Glucosidic Linkage.—Laminarin (18 g.) was hydrolysed with oxalic acid (400 c.c.), as described above. After 3 hours' heating the solution was neutralised with chalk, and emulsin was added in small quantity to the filtrate. The rotation of the solution gradually increased from + 2·38 to + 3·70° in 7 days. No laminaribiose could now be detected in the liquid. In other experiments, laminarin was partially hydrolysed by oxalic acid, and after neutralisation with chalk the glucose was destroyed by yeast. Addition of emulsin to this liquid after removal of yeast caused glucose to be produced in considerable quantity.

The author wishes to express his gratitude to Professor Dillon for the interest he has taken in this work, to Professor S. Shea, M.D., for the photomicrograph of the crystalline osazone, and to the Royal Dublin Society for the loan of a polarimeter. In conclusion, grateful acknowledgment is made to the Industrial Research Council of Ireland, under whose auspices this work was carried out.

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No. 45.

REPORT OF THE IRISH RADIUM COMMITTEE FOR THE YEAR 1940

[Published separately 26TH AUGUST, 1941.]

1,037 tubes containing 8,571 millicuries of radon were issued during 1940, as compared with 1,160 tubes containing 8,630 millicuries in 1939.

The new glass apparatus used in the preparation of these tubes and constructed for the Society in 1939 by Mr. W. Brady in the Chemistry Department of University College, Dublin, has proved very satisfactory in use.

Mr. F. S. Stewart, B.A., was appointed to the Radium Exhibition falling vacant during the year.

Returns from the chief users record the treatment of the following cases with radon or radium element during 1940:—

MALIGNANT CASES.

Dr. Oswald Murphy, St. Vincent's Hospital, Dublin ...	91
Dr. Oliver Chance, Richmond, Sir Patrick Dun's, Dr. Steevens', Rotunda, and Coombe Hospitals, Dublin	14
Dr. R. E. Tottenham, City and County Hospital, Londonderry	5
St. Anne's Hospital, Dublin	163
City and County Infirmary, Waterford	19
Galway Central Hospital	8
St. John's Hospital, Limerick	8
Total, ...	308

NON-MALIGNANT CASES.

Dr. Oswald Murphy	80
Dr. Oliver Chance	18
Dr. R. E. Tottenham	3
National Maternity Hospital, Dublin	1
Total, ...	102

The returns show that for the cases of malignant disease first seen during 1940 the numbers treated by the different methods were as follows:—

No. of cases treated	...	...	...	991
„ „ „	by Surgery alone	...	...	142
„ „ „	by Radium alone	...	...	223
„ „ „	by X-rays alone	...	...	505
„ „ „	by Surgery and Radium	...	...	20
„ „ „	by Surgery and X-rays	...	...	36
„ „ „	by Radium and X-rays	...	...	63
„ „ „	by Surgery, Radium, and X-rays		...	2

The following reports have been received, summarising the results of treatments carried out with Radium or Radon (or in some cases X-rays) during the year:—

Report by Oswald J. Murphy, M.B., B.Ch.,

St. Vincent's Hospital, Dublin.

Malignant Cases.

Treated by Surgery and Radium	...	...	4
Treated by Radium and X-rays	...	...	8
Treated by Radium only ...	...	...	79
			91

Carcinoma of the ear, 1. No glands, 1. Improved, 1.
Carcinoma of the lip, 12. No glands, 9. Improved, 1. Apparently well, 8.
Carcinoma of the cheek, 2. No glands, 2. Improved, 2.
Carcinoma of the parotid, 1. No glands, 1. Improved, 1.
Carcinoma of the tongue, 4. No glands, 0. Anterior third, 1. Well locally. Middle third, 2. Improved, 1. Died, 1. Posterior third, 1. Improved, 1.
Carcinoma of floor of the mouth, 1. No glands, 0. Improved, 1.
Carcinoma of the bladder, 2. Improved, 1. Apparently well, 1.
Carcinoma of uterine body, 1. Apparently well, 1.
Carcinoma of uterine cervix, 7. Group I, 5. Apparently well, 4. Group II, 2. Apparently well, 1. Improved, 1.
Carcinoma of the skin surfaces, 19. No glands, 19. Improved, 6. Well, 13.
Rodent ulcers, 41. Well, 28. Improving, 12.

NON-MALIGNANT CASES.

Hodgkin's disease, 2. T.B. cervical glands, 6. T.B. peritonitis, 8. Uterine hæmorrhage, 2. Angiomata, 20. Lupus vulgaris, 3. Lupus Erythematosus, 1. Keloids, 4. Dupeytron contraction, 1. Pruritis vulvae, 1. Warts, 32. Total, 80.

Report by Oliver Chance, M.B., B.Ch., D.M.R.E.,

Richmond, Sir P. Dun's, Dr. Steevens', Rotunda, and Coombe Hospitals.

During 1940 most of the cases requiring radiation seen in the above hospitals were referred to St. Anne's Hospital for treatment. The bulk of the cases included below were either private patients or else suffering from such conditions as Rodent Ulcers for which they could be treated as out-patients.

Altogether 132 malignant patients and 41 patients suffering from benign conditions were treated by radiation.

The following is a list of the cases in which radon was used:—

MALIGNANT.

Carcinoma cervix uteri	2
Carcinoma body of uterus	1

BENIGN.

Toxic goitre	9
T.B. peritonitis	6
Menopausal hæmorrhage	2
Naevus	1

The increasing use of Contact X-rays in cases of superficial malignant disease is largely responsible for the great decrease in the amount of radon used.

Report by R. E. Tottenham, M.D., B.Ch., B.A.O.,

City and County Hospital, Londonderry.

I only see the cases of uterine cancer, and those occurring in other parts of the body do not come under my care. The following five cases were treated:—

Mrs. McK.—Carcinoma of the cervix.

Three applications as follows:—1st, 2,394 M.E.H. 2nd, 1,729 M.E.H. 3rd, 2,793 M.E.H. Total, 6,916 M.E.H.

For about six months her condition was satisfactory. She then developed an abscess in the lower abdomen, which had to be opened. Her health at the

moment (July, 1941) is very poor (i.e. 1½ years after treatment). She also appears to have an old standing tubercular lesion.

Mrs. C. D.—Carcinomatous ulcers of vulva.

This patient received in all about 2,000 M.E.H. in interrupted periods, the radon being applied in needles. The results were not very satisfactory.

Mrs. M.—Columnar celled carcinoma of cervix.

Initial course of approximately 8,000 M.E.H. Condition showed improvement for a short time. Five months later there was a recurrence.

A second course of smaller dosage was given, which was followed by improvement for about three months, when more recurrences appeared. The patient has since died.

In my experience columnar celled carcinomas are not very radio sensitive.

I have lost trace of the other two cases who have gone to the country; they were both columnar celled type.

I have used radon with some success in the treatment of non-malignant hæmorrhages of middle age, the radon capillaries being inserted into a round brass container about an inch and a half long. This container has thick walls, and is about the diameter of the cap of a fountain pen. It is pushed up into the uterus.

Report from St. Anne's Hospital, Dublin.

Radiologist: Dr. Oliver Chance.

163 cases were treated with radon or radium element. Of 41 cases of carcinoma of cervix uteri seen at above hospital a total of 36 were treated, viz., 12 with radium or radon alone, 4 with X-rays alone, and 20 with radium and X-rays combined. 17 are known to be alive, 4 are known to be dead, and 15 cases are untraced.

Report from St. John's Hospital, Limerick.

Radiologist: H. G. Roche Kelly, M.D., D.P.H.

Number of cases treated during the year 1940, 8. All these were cases of rodent ulcer of the face situated on the cheek, temple, forehead, or eyelid. Treatment was by insertion of seeds in margin (4 cases) or by surface application in applicators built up from adhesive plaster (4 cases). In all cases complete healing resulted.

VOLUME 22.

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